Molecular Genetics and Metabolism 73, 259–267 (2001)
doi:10.1006/mgme.2001.3185, available online at http://www.idealibrary.com on IDEAL®

Impairment of Type III Group B *Streptococcus*-Stimulated Superoxide Production and Opsonophagocytosis by Neutrophils in Diabetes[1]

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Received December 6, 2000, and in revised form March 22, 2001; published online June 13, 2001

The effect of hyperglycemia upon susceptibility to bacterial infection in diabetes mellitus is incompletely elucidated. The present experiments assessed the effect of hyperglycemia upon neutrophil-mediated phagocytosis of type III group B *Streptococcus* (GBS). Type III GBS was chosen for study because the incidence of invasive GBS disease is substantially increased in type 2 diabetic compared with nondiabetic subjects. The hypothesis tested was that severe hyperglycemia would alter neutrophil metabolism by diverting NADPH from superoxide production into the aldose reductase-dependent polyol pathway that converts glucose into sorbitol and thus would impair opsonophagocytosis (OP) of type III GBS. Neutrophils from 10 adults with type 2 diabetes had no intrinsic phagocytic defect under baseline glycemic conditions. After equilibration in 60 or 120 mM glucose or in 60 mM choline chloride, OP activity was reduced significantly ($P \leq 0.03$). Neutrophil superoxide production correlated with glucose concentration and also was significantly reduced during hyperglycemia ($P < 0.05$). Addition of III GBS capsular polysaccharide-specific IgG in a sufficient concentration supported efficient OP, even during hyperglycemia. Alrestatin, an aldose reductase inhibitor, increased superoxide production and significantly improved OP of type III GBS ($P = 0.03$). Thus, diversion of NADPH into the polyol pathway is one mechanism by which OP of GBS III is impaired during hyperglycemia, and this effect is mitigated when levels of capsular polysaccharide-specific IgG are sufficient.

Key Words: **diabetes; group B *Streptococcus;* superoxide; aldose reductase; polyol pathway; pathogenesis.**

Group B streptococcal (GBS) infections cause substantial morbidity and mortality among adults. The manifestations of infection include necrotizing fasciitis and toxic shock-like syndrome, soft tissue infection, osteomyelitis, meningitis, pneumonia, peritonitis, and endocarditis (1–8). Underlying medical conditions in adults who develop GBS disease include diabetes mellitus, cirrhosis and other chronic liver diseases, malignancy, and immunosuppression (9–11). In a population-based study in metropolitan Atlanta, adults with diabetes had a 10.5-fold higher risk (CI 7.8 to 14.4) than the general population for invasive GBS disease (12). Assessments of vaginal GBS colonization rates among women with diabetes mellitus have yielded inconsistent results (13–15), suggesting that increased mucosal carriage with the organism is not an explanation for enhanced susceptibility. Some investigators have suggested that altered polymorphonuclear leukocyte (PMNL) phagocytosis and killing is a major factor enhancing the invasive potential of GBS in these patients as has been shown for infection with *Candida albicans* (16) and *Escherichia coli* (17). Others, referencing stud-

[1] Presented in part at the 37th annual meeting of The Infectious Diseases Society of America, November 1999, Philadelphia, PA (Abstract No. 178) and at the Pediatric Academic Societies 2000 Annual Meeting, May 2000, Boston, MA (Abstract No. 2027).

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1096-7192/01 $35.00

ies showing that critical PMNL functions are disrupted only with severe hyperglycemia, suggest that PMNL dysfunction is only of minimal concern, and that other factors contribute more substantially to increased susceptibility (18).

The present study tested the hypothesis that metabolic compensatory mechanisms for maintaining cell size and volume during extreme hyperglycemia would impair phagocytosis and killing of type III GBS. If a functional impairment was demonstrated, we reasoned that the mechanism would be that NADPH was diverted from superoxide (O_2^-) production into the aldose reductase-dependent polyol pathway that converts glucose into sorbitol, resulting in an overall reduction in GBS-stimulated O_2^- production by these stressed cells. To test this hypothesis, we examined the extent to which hyperglycemia affected PMNL-mediated killing of type III GBS, and the effect of GBS III capsular polysaccharide-specific IgG (GBS III CPS-specific IgG) upon these PMNL functions during hyperglycemia. The opsonophagocytosis (OP) of GBS III by PMNL from diabetic and nondiabetic subjects was examined at the physiologic limit and under pharmacologic conditions of hyperglycemia with the goal that, if OP were reduced substantially under these conditions, experiments to further quantify the effect at lower more moderate levels of hyperglycemia would be conducted.

The present experiments documented the existence of a hyperglycemia-induced attenuation of O_2^- production that resulted in inefficient PMNL-mediated phagocytosis and killing of type III GBS. Inhibition of aldose reductase, an enzyme that competes for NADPH, effected significant reversal of this impairment in PMNL metabolism. This defect had little impact upon efficiency of GBS killing when PMNL were provided exogenously with a sufficient concentration of GBS III CPS-specific IgG. Thus, attention to glucose homeostasis and development of GBS vaccines theoretically could lower the risk of invasive GBS disease in diabetes.

MATERIALS AND METHODS

Subjects. A preenrollment medical history was obtained for each diabetic subject who volunteered for this study. Informed consent was obtained from patients and human experiment guidelines of the U.S. Department of Health & Human Services and those of the Baylor College of Medicine Institutional Review Board for Human Research were followed in the conduct of this clinical research. Each subject had been given the diagnosis of type 2 diabetes mellitus by his or her physician. Nondiabetic subjects were healthy laboratory volunteers. All subjects were free of known infection at the time of evaluation of PMNL function.

Determination of blood glucose. At phlebotomy, glucose concentrations were performed with an Exactech RSG Glucometer (Medisense, Bedford, MA).

PMNL isolation. Leukocyte-rich plasma was collected from anticoagulated whole blood after sedimentation. Following centrifugation at 300*g*, the cellular fraction was resuspended with minimal essential media (MEM) with Earle's salts and 5.5 mM glucose (GIBCO BRL, Life Technologies, Rockville, MD). PMNL were purified by centrifugation through 5 ml of Ficoll-Hypaque (Ficoll, Sigma Chemical Co., St. Louis, MO; Hypaque, Nycomed, Princeton, NJ) at 700*g* for 20 min and rinsed in MEM. The remaining erythrocytes were removed by hypotonic lysis. PMNL were resuspended in MEM and brought to a concentration of 10^7 PMNL/ml. Purity was greater than 98% PMNL at the end of isolation. The viability of PMNL, determined by Trypan blue staining at the conclusion of the experiments, exceeded 92%.

Equilibration. PMNL (1.5×10^6) were equilibrated for 90 min at 4°C in 220 μl of MEM with the addition of D-glucose to mimic the glucose concentration at phlebotomy or at glucose concentrations of 5.5, 60, or 120 mM. The 60 mM (1080 mg/dl) glucose concentration was chosen to correspond with the upper range of the physiologic limit reported to occur during diabetic ketoacidosis (19). The 120 mM glucose concentration was chosen to provide a pharmacologic condition for the purpose of accentuating any subtle defect in neutrophil metabolism that might contribute to dysfunction at more modest levels of hyperglycemia. Some PMNL were also equilibrated in MEM with 60 mM choline chloride (Calbiochem, La Jolla, CA). Choline chloride, which has been used experimentally to reduce intracranial pressure in lieu of mannitol, is an efficient, organic, GBS-nonnutritive osmolyte resulting in at least 2 osmol of osmotic stress for every 1 osmol generated by glucose (20). This osmolyte was chosen for comparative purposes since it does not promote GBS III growth *in vitro*. By contrast, improved growth and capsule formation of GBS occur when the growth media is supplemented with glucose.

Opsonophagocytic assay. PMNL from diabetic and healthy adults were tested in parallel using an assay described previously (21), with the following modifications. After equilibration of PMNL, 60 μl of serum and 20 μl of early log-phase type III GBS, strain COH1 (2–3 × 10^6 CFU), were added to the reaction mixture. Each assay included control sera containing a high and a low concentration of GBS III CPS-specific IgG and a PMNL-free control. Aliquots of the PMNL and GBS-containing reaction mixture were removed at 0 min and after 60 min of incubation at 37°C. The PMNL were lysed in sterile water and dilutions of the aliquots in saline were plated to determine the CFU. For some experiments, additional time intervals were included. OP activity, reflecting PMNL-mediated phagocytosis and killing, was expressed as the $\log_{10}$ ± standard error of the mean (SEM) reduction in CFU, calculated as $\log_{10}$ ([CFU at 0 min]/[CFU at 60 min]) ± SEM.

Antibody determinations. Serum from diabetic or nondiabetic subjects was processed to preserve endogenous complement activity. The concentration of GBS III CPS-specific IgG was determined by ELISA, as previously described (22,23).

Determination of glycosylated hemoglobin. The percentage of total glycosylated hemoglobin was determined by high-performance liquid chromatography using the Primus CLC33 (Kansas City, MO). The percentage of hemoglobin A_{1C} (HbA_{1C}) was calculated from that value.

Measurement of superoxide production. The measurement of O_2^- was modified from a previously described method (24). Sixty microliters of human serum with a concentration of GBS III CPS-specific IgG of 0.1 μg/ml was added to 200 μl of equilibrated PMNL from diabetic subjects (1.5 × 10^6). O_2^- production was measured by a modification of the lucigenin-enhanced chemiluminescence method (25). Five minutes after addition of 20 μl of lucigenin, 1.25 × 10^5 M (F.W. 510.5 g/mol, Sigma Chemical Co.), 20 μl of log-phase GBS III, strain COH1 (2–3 × 10^6 CFU), was injected. GBS-stimulated chemiluminescence, measured in relative light units (RLU) and corrected for autochemiluminescence, was recorded at 5-min intervals using a TD-20/20 Luminometer (Turner Designs, Sunnyvale, CA). Settings included a delay time of 0 s and an integrate time of 10 s, with a single replicate.

Aldose reductase inhibitors. The aldose reductase inhibitors, statil (MW 391.2 g/mol), alrestatin (MW 255.22 g/mol), and 4-naphthalimidobutyric acid (MW 283.27 g/mol), were obtained from Tocris Cookson Inc. (Ballwin, MO). The dry compounds were suspended in 100% DMSO then diluted 100-fold in MEM. The solutions were added to the glucose-adjusted cell media prior to addition of diabetic PMNL. The aldose reductase inhibitors were evaluated at 11 or 22 μM concentrations for the period of osmotic equilibration, while DMSO composed 0.1% of the reaction mixture. Selection of these concentrations was based upon a report indicating that a daily dose of 300 mg of statil, given orally, results in an average plasma level of 11 μM (26).

Statistical analysis. The effect of GBS III CPS-specific IgG and glucose concentration on OP activity was tested in a randomized block (subject) design with a two-way treatment structure. Replicated measures within subjects for each antibody-glucose combination were averaged prior to statistical analysis. Analysis of variance for repeated measures (ANOVA-RM), as provided by the SPSS Inc. general linear model analysis, was used to simultaneously test the effects of antibody, glucose, diabetic status, and interactions among these factors. The results represent the means ± SEM of three to five experiments, unless otherwise stated. Levels of significance for comparisons between samples were determined using Student's paired t test (two-tailed). P values < 0.05 were considered significant. One-way analysis of variance, followed by Dunnett's multiple comparison test, as provided by the Graphpad Prism statistical program, was used to determine the effect of hyperglycemic stress on superoxide production.

RESULTS

Subjects. The 10 diabetic subjects ranged in age from 32 to 65 (mean 48) years and had the diagnosis of type 2 diabetes for a mean of 5 years (range 2–10). Except for one subject controlled by diet alone, all were taking one or more oral hypoglycemic agents. One subject required insulin injections in addition to oral hypoglycemic agents. Nondiabetic subjects were healthy adults ranging in age from 28 to 37 years. The mean phlebotomy glucose concentrations for diabetics averaged 13 mM (range 7.6–18.3) or 235 mg/dl (range 137–330), while that for nondiabetic subjects was 4.5 mM (range 2.3–5.4) or 81 mg/dl (range 42–98).

OP activity of unstressed PMNL. We first sought to assess the intrinsic phagocytic and killing capac-

TABLE 1
Opsonophagocytic (OP) Activity of PMNL from Diabetic and Nondiabetic Subjects

Source of PMNL	Number of subjects	Mean (range) glucose concentration (mM)	Log_{10} (±SEM) OP activity at GBS III capsular polysaccharide (CPS)-specific concentration shown		
			13 μg/ml*	0.1 μg/ml*	0.05 μg/ml*
Nondiabetic subjects	7	4.5 (2.3–5.4)	2.05 (0.09)	1.56 (0.13)†	0.62 (0.12)§
Diabetic subjects	10	13 (7.6–18.3)	2.01 (0.08)	1.43 (0.09)†	0.65 (0.1)§

* P = NS for OP activity mediated by PMNL from nondiabetic versus diabetic subjects.
† $P \leq 0.01$ comparing OP activity at 13 μg/ml vs 0.1 μg/ml of GBS III CPS-specific IgG.
§ $P \leq 0.001$ comparing OP activity at 13 μg/ml vs 0.05 μg/ml of GBS III CPS-specific IgG.

ity of PMNL from diabetic subjects for type III GBS. As shown in Table 1, the mean OP activity was similar for PMNL from the 10 diabetic and 7 nondiabetic subjects when tested in the presence of normal exogenous serum containing each of three concentrations of GBS III CPS-specific IgG, 13, 0.1, and 0.05 μg/ml. These antibody levels were chosen based on previous observations demonstrating consistent efficient *in vitro* functional activity (≥1 log_{10} reduction in CFU) for sera with GBS III CPS-specific IgG exceeding 0.5–1.0 μg/ml and variable but often lower functional activity (<1 log_{10} reduction in CFU) for sera with <0.5 μg/ml of III CPS-specific IgG (21). The sera with 0.1 and 0.05 μg/ml were chosen as representative of low antibody-containing sera with efficient and inefficient function, respectively. OP activity by PMNL from diabetic and nondiabetic subjects was significantly greater when tested with serum containing 13 μg/ml of GBS III CPS-specific IgG added to the reaction mixture than with either of the two lower antibody concentrations. The finding that there was no significant difference in OP by PMNL from diabetic or nondiabetic subjects at any concentration of CPS-specific IgG tested excluded an intrinsic defect of diabetic PMNL-directed OP against GBS III.

OP activity of PMNL during hyperglycemia. The next set of experiments was designed to determine the effects of hyperosmolarity per se and hyperglycemia, in particular, upon PMNL-mediated phagocytosis and killing of type III GBS in diabetes. OP activity for PMNL from the 10 diabetic subjects against GBS III using endogenous sera decreased with increasing glucose concentration or when choline chloride was employed (Fig. 1). OP activity at the phlebotomy glucose concentration (1.41 ± 0.27 log_{10} CFU) was significantly greater than that for PMNL equilibrated for 90 min in 60 mM glucose (1.14 ± 0.22), 120 mM glucose (0.92 ± 0.19), or 60 mM choline chloride (0.69 ± 0.16 log_{10} CFU) (P = 0.025, 0.002, and 0.002, respectively). When PMNL from diabetic subjects were equilibrated in 5.5 mM glucose, the OP activity (1.51 ± 0.27 log_{10} CFU) did not differ significantly from that at the phlebotomy glucose concentration. These results suggested that changes in osmolarity mediate the observed effect seen with hyperglycemia.

Influence of GBS III CPS-specific IgG on OP. The concentration of GBS III CPS-specific IgG in serum correlates significantly with functionality *in vitro* measured by OP (22). Thus, we sought to determine whether a high concentration of GBS III CPS-specific IgG negated the observed effects of hyperosmo-

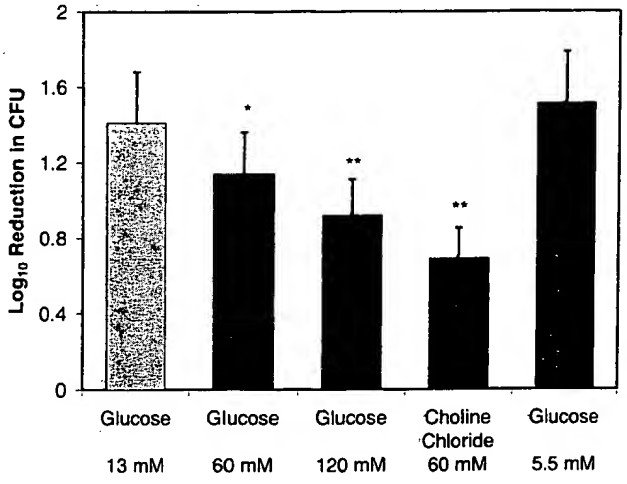

FIG. 1. Effect of osmotic stress on OP activity for type III GBS, strain COH1, by PMNL from 10 diabetic subjects. OP activity at the phlebotomy glucose concentration (mean 13 mM; range 7.6–18.3) is shown in the stippled bar. The median endogenous serum GBS III CPS-specific IgG concentration was 1.6 μg/ml (range 0.2–37.1 for these 10 sera). *P = 0.025 compared with 13 mM glucose; **P = 0.002 compared with 13 mM glucose.

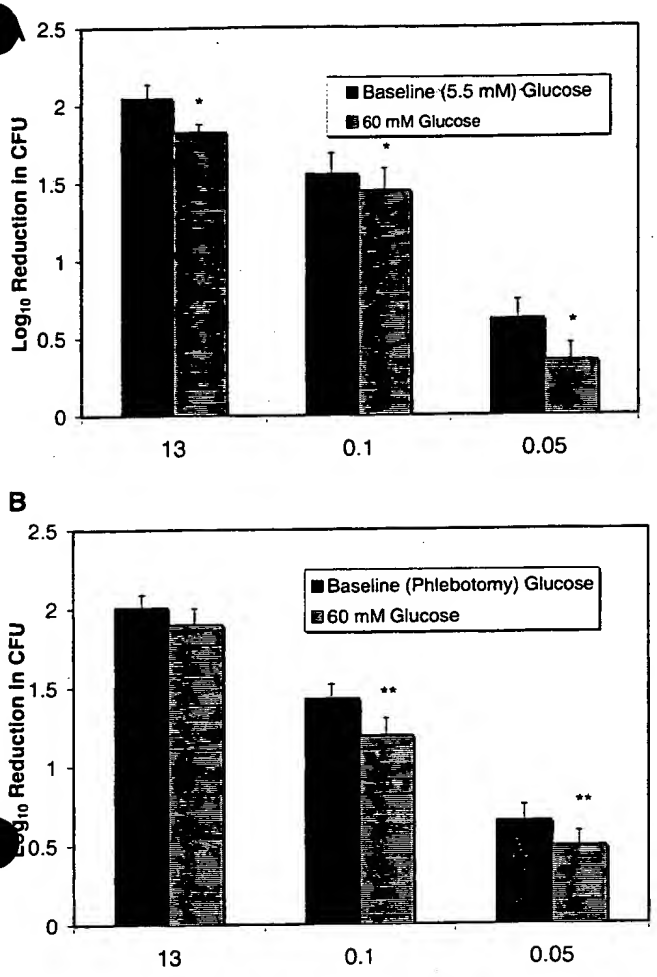

FIG. 2. Influence of GBS III CPS-specific IgG concentration (μg/ml) on OP activity of nondiabetic (A) and diabetic (B) subject PMNL against type III GBS. OP activity was determined for both osmotically stressed and unstressed PMNL at each of three serum concentrations of exogenous GBS III CPS-specific IgG (13, 0.1 or 0.05 μg/ml). Unstressed PMNL (solid black bars) were equilibrated with the baseline glucose concentration (5.5 mM for nondiabetic subject PMNL) or to the phlebotomy concentration (range 7.6–18.3 mM) for diabetic subject PMNL. Stressed PMNL (shaded bars) were equilibrated with 60 mM glucose. *$P \leq 0.05$, 60 mM glucose vs baseline; **$P \leq 0.005$, 60 mM glucose vs baseline.

larity. PMNL were tested using the same 90-min glucose equilibration model above, with or without equilibration in 60 mM glucose, and with the sera containing 13, 0.1, or 0.05 μg/ml of GBS III CPS-specific IgG. The phagocytosis and killing portion of these experiments also was conducted at a 60 mM glucose concentration. A high concentration of GBS III CPS-specific IgG failed to protect against a hyperglycemia-induced reduction in OP activity (Fig. 2). The OP activity for PMNL from nondiabetic subjects declined from 2.05 ± 0.09 to 1.83 ± 0.05 $\log_{10}$ CFU ($P = 0.05$) using 13 μg/ml antibody serum, though efficient (>1 $\log_{10}$) reduction in GBS colonies was still achieved. For PMNL from diabetic subjects, OP activity also decreased from 2.01 ± 0.08 to 1.90 ± 0.10 $\log_{10}$ CFU using 13 μg/ml antibody serum (P = NS), and was significantly diminished when tested with serum containing 0.1 or 0.05 μg/ml of GBS III CPS-specific IgG ($P = 0.005$ and 0.004, respectively).

Use of serum deficient in GBS III CPS-specific IgG (0.05 μg/ml) resulted in the greatest impairment of OP activity for both diabetic and nondiabetic PMNL. When simultaneously testing the effects of diabetic status, GBS III CPS-specific IgG concentration, hyperglycemic stress, and interactions among these factors by ANOVA-RM, no statistically significant two or three-factor interactions were detected. This indicated that the effect of GBS III CPS-specific IgG concentration ($P < 0.001$) and the effect of hyperglycemia ($P < 0.001$) were independent of each other and of diabetic status. Thus, there was no specific impairment intrinsic to PMNL from diabetic subjects. Rather, the effect of increasing glucose occurred with PMNL regardless of subject origin and was not abolished by a high concentration of GBS III CPS-specific IgG. However, the efficiency of OP activity outweighed the magnitude of the reduction in OP activity induced by hyperglycemic stress (Fig. 2).

Effect of percentage hemoglobin A_{1C} on OP. We had hypothesized, based upon the observations of MacRury *et al.* (27), that *in vitro* functional activity for GBS III by diabetic sera would be related inversely to the percentage of HbA_{1c} in sera. Poor long-term diabetes glucose control was shown to correlate with impaired PMNL phagocytic function. Thus, the HbA_{1c} percentage was determined for the diabetic subjects in the present study and its correlation with OP activity determined. The mean percentage HbA_{1C} was 8.9% and the values ranged from 6.6 to 12.2%. A modest inverse relationship between OP activity and the percentage HbA_{1C} was found that was not statistically significant (results not shown). The correlation between better long-term glycemic control and improved OP activity was conserved under differing glucose and GBS III CPS-specific IgG concentrations.

Correlation of GBS-stimulated superoxide production with OP activity against GBS. Experiments next questioned whether O_2^- production correlated quantitatively with OP activity. The ratio of O_2^- pro-

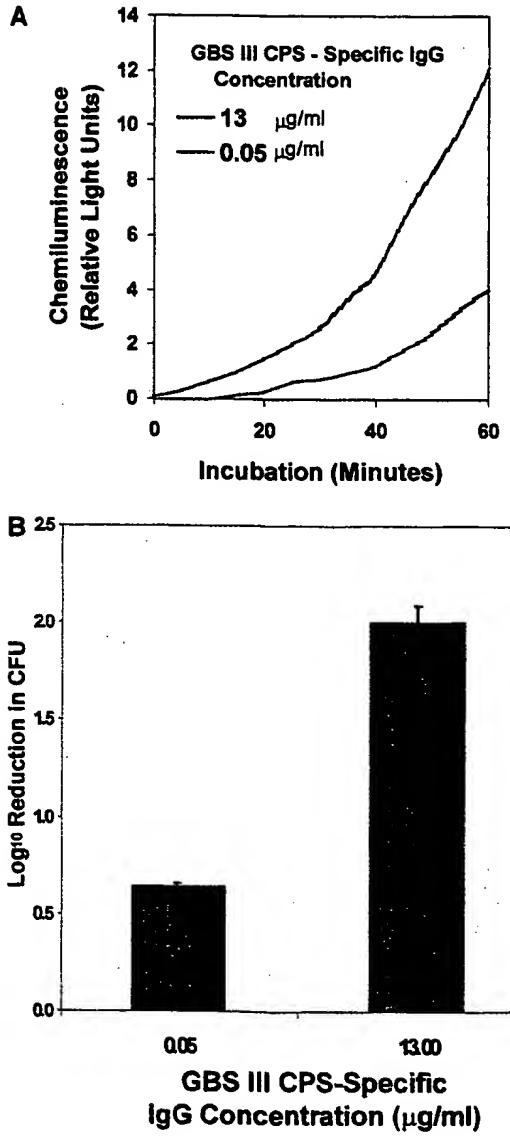

FIG. 3. Effect of endogenous serum concentration (µg/ml) of GBS III CPS-specific IgG on live GBS-stimulated superoxide production (A) and OP activity (B).

duction after 60 min of stimulation with GBS III for PMNL in the low (0.05 µg/ml) concentration of GBS III CPS-specific IgG to that for PMNL in the presence of the high (13 µg/ml) concentration of GBS III CPS-specific IgG was similar to the ratio of OP activity after 60 min for PMNL from the seven nondiabetic subjects tested under the same conditions (Fig. 3). These results suggested that GBS-stimulated chemiluminescence was a CPS-specific IgG-dependent phenomenon. Thus, O_2^- production and OP activity maintain quantitative relationships based upon the amount of antibody in the reaction.

Superoxide production of hyperglycemic-stressed diabetic PMNL. Next, experiments were designed to determine whether the mechanism of diminished OP related to decreased O_2^- production. O_2^- production by diabetic PMNL from several of the donors for the experiments above diminished with increasing glycemic and osmotic stress (equilibration in 60 and 120 mM glucose, or 60 mM choline chloride, respectively) compared to diabetic subject PMNL equilibrated with 5.5 mM glucose (Fig. 4). When the area under the curve for each reaction condition using one-way analysis of variance (Dunnett's multiple comparison test) was compared, the means were significantly different ($P = 0.0024$). Specifically, compared to PMNL equilibrated in 5.5 mM glucose, the area under the curve was significantly less for PMNL equilibrated in 120 mM glucose ($P < 0.05$), or choline chloride ($P < 0.01$). The finding of a greater effect of choline chloride on PMNL functions compared to sugars such as sucrose has been reported by others (28). In our experiments there was no disparity in survival for PMNL equilibrated in choline chloride or glucose to explain these results. Using serum with 0.1 µg/ml of GBS III CPS-specific IgG, O_2^- production was attenuated by increased glycemic stress in a manner that correlated well with reduced OP activity. Thus, hyperglycemia-induced reduction in OP activity is associated with a similar stepwise reduction in O_2^- production (see Figs. 1 and 4).

Inhibition of aldose reductase in hyperglycemic-stressed PMNL. Finally, since diversion of NADPH to the polyol pathway was a potential mechanism for

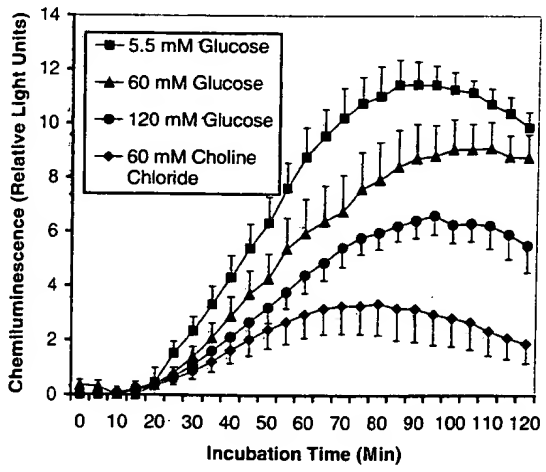

FIG. 4. Effect of osmotic stress on lucigenin-enhanced chemiluminescence of diabetic subject PMNL stimulated with live GBS.

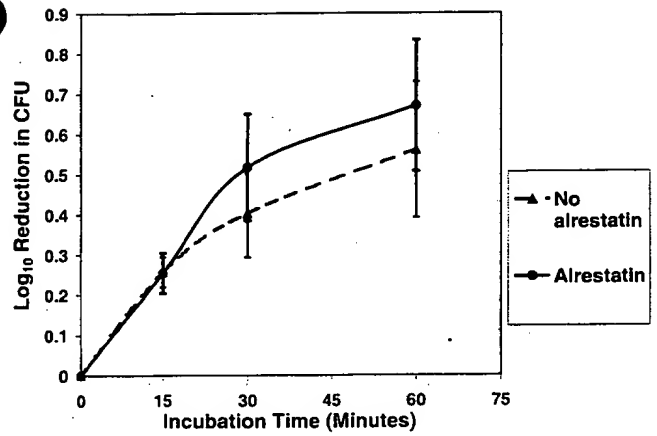

FIG. 5. Effect of aldose reductase inhibition on OP of GBS III by PMNL from diabetic subjects. PMNL were equilibrated with 120 mM glucose with or without alrestatin for 90 min at 4°C and OP activity at 37°C was determined at the timed intervals shown.

reduced O_2^- production, experiments were designed to block the polyol pathway. We hypothesized that this should increase O_2^- production and, thus, OP activity. No substantial differences in effect upon O_2^- production were noted when several aldose reductase inhibitors were compared in preliminary experiments (data not shown). Alrestatin at the 11 μM concentration reported to be physiologically achievable (26) effected a 27% increase in O_2^- production over baseline and was chosen as a representative aldose reductase inhibitor for subsequent OP experiments. A time-dependent improvement in OP activity was observed for diabetic PMNL which had equilibrated in 120 mM glucose and alrestatin, and this was significant ($P = 0.03$) when OP was assessed after both 30 and 60 min incubation (Fig. 5). The increase in OP activity with alrestatin inhibition demonstrates that blocking the polyol pathway leads to increased O_2^- production and contributes significantly to improved killing of GBS III by PMNL from diabetic subjects during hyperglycemic stress.

DISCUSSION

The extent to which a metabolic impairment in PMNL function may contribute to impaired immunity in diabetes mellitus is a complex issue (27,29,30). The contributions of organism-specific virulence factors, diabetic serum factors, and function of the antibody molecule in diabetes all are potential variables in the host-pathogen interaction (31,32). GBS is an important pathogen in diabetes but the pathogenesis of infection is largely unstudied. We found that in the absence of acute hyperglycemia, PMNL from diabetic subjects receiving oral hypoglycemic agents functioned as well as those from nondiabetic subjects in PMNL-mediated killing of type III GBS. This finding could reflect correction of some deficiencies in function by the patients' treatment for hyperglycemia. Improved PMNL phagocytic function for diabetic subjects during periods of good glycemic control has been demonstrated by MacRury and colleagues (27). Alexiewicz *et al.* (33) found that when patients with newly diagnosed type 2 diabetes were treated with glyburide, the average percentage HbA_{1C} fell from 15.8 to 8.4% within 3 months, and deficient PMNL-mediated phagocytosis of oil red O-containing *E. coli* lipopolysaccharide-coated oil droplets improved to values approaching those measured for control PMNL (33).

In contrast, PMNL-mediated OP of type III GBS was impaired under *in vitro* conditions simulating an episode of acute severe hyperglycemia. The reduction in OP was inducible for PMNL from both diabetic subjects and nondiabetic controls and it correlated with attenuation of O_2^- formation in hyperglycemia-stressed PMNL. Other investigators also have identified PMNL-associated microbial defects in association with lower glucose concentrations, but these may be organism-specific. For example, measurable deficiencies in *C. albicans* killing by PMNL from healthy adults have been observed at a glucose concentration as low as 10 mM with killing abolished at 50 mM (16,25).

The mechanism underlying the reduction in PMNL-mediated bactericidal activity for GBS III, suggested by experiments with choline chloride, involves a compensatory metabolic pathway that maintains osmotic neutrality. Demerdash *et al.* (28) have shown that choline chloride generates an osmotic effect similar to that produced by a higher concentration of glucose and causes a similar effect quantitatively on intracellular calcium concentration. In the osmolar stress of hyperglycemia, there are demands upon stores of NADPH, a resource required for initiation of the respiratory burst. The intracellular conversion of glucose to sorbitol, which helps to maintain cell volume, requires the NADPH-dependent enzyme, aldose reductase, and is effected through the polyol pathway. Increased demand for NADPH shunts available NADPH away from NADPH oxidase, an enzyme involved in O_2^- production. Accordingly, hyperglycemia-stressed PMNL

have reduced O_2^- production and are sorbitol-rich (31). This inducible reduction in O_2^- production is well-conserved, occurring whether stimulation is induced by a chemotactic peptide, calcium ionophore, phorbol ester, or by the Fc portion of IgG (34). Our experiments with choline chloride also revealed an inducible reduction in O_2^- production. Although the compensatory metabolic pathways that maintain osmotic neutrality may differ (20), the results of the choline chloride experiments suggest that hyperosmolarity per se causes the reduction in PMNL-mediated bactericidal activity.

The NADPH depletion mechanism has been proposed by others who found that when diabetic subjects were treated with the aldose reductase inhibitor, tolrestat, for 4 weeks phorbol myristate acetate-stimulated PMNL respiratory burst activity improved (35). Similarly, better killing of *E. coli* by PMNL from diabetic subjects was noted with ponalrestat after 12 weeks of treatment (17). Our *in vitro* experiments suggest that aldose reductase inhibition reverses the severe hyperglycemia-induced attenuation of GBS-stimulated superoxide production.

Another explanation for hyperglycemic PMNL dysfunction involves accumulation of cytoplasmic calcium that occurs during osmotic equilibration (28). High PMNL cytoplasmic calcium concentration may impair phagocytosis, cause decreased calcium-dependent intracellular signaling in response to stimuli, and may inhibit intracellular ATP production and prevent calcium export (33,36,37). Normalization of cytoplasmic calcium does not fully correct defects in phagocytosis under conditions of experimental infection (38). These findings suggest that more than one factor may contribute to reduced killing of GBS. Other aspects of immune dysfunction, such as poor wound healing and inadequate clearance of GBS bacteremia by the reticuloendothelial system (39), also may increase susceptibility to GBS disease in diabetes.

Even after equilibration in high concentrations of glucose, OP activity of diabetic PMNL remains efficient when the concentration of GBS CPS-specific IgG is at or above the concentration proposed to correlate with protection from invasive infection (C. J. Baker *et al.*, manuscript submitted for publication). This occurs because the magnitude of GBS III killing is greater than that of the hyperglycemia-induced reduction in OP activity. For this reason, immunization of diabetic adults against GBS using a polyvalent GBS capsule conjugate vaccine may preserve efficient OP against GBS, regardless of the degree of sudden glycemic shifts. Nonetheless, though we have shown hyperglycemia to reduce PMNL-mediated phagocytosis and killing of GBS, and reduced superoxide production may be intermediary, the extreme serum glucose concentrations required to alter PMNL killing would argue against a major role of acute hyperglycemia shifts in the pathogenesis of invasive infection in diabetes by GBS in particular.

ACKNOWLEDGMENTS

The authors thank Dr. Ching-Nan Ou, Ph.D., and the Department of Pathology at Texas Children's Hospital for performing glycosylated hemoglobin measurements; Claire M. Skeeter and Melissa E. Hickman for excellent technical assistance; E. O'Brian Smith, Ph.D., for assistance with statistical analysis; Robin Schroeder for help with manuscript preparation; and Carol J. Baker, M.D., for helpful suggestions. This work was supported in part by The Streptococcal Initiative, Contract N01 AI 75326, from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. Dr. Mazade was supported by the Pfizer Fellowship Award of the Pediatric Infectious Diseases Society.

REFERENCES

1. Azzam ZS, Ron Y, Oren I, Sbeit W, Motlak D, Krivoy N. Group B streptococcal tricuspid valve endocarditis: A case report and review of the literature. *Int J Cardiol* **64**:259–263, 1998.
2. Domingo P, Barquet N, Alvarez M, Coll P, Nava J, Garau J. Group B streptococcal meningitis in adults: Report of twelve cases and review. *Clin Infect Dis* **25**:1180–1187, 1997.
3. Farley MM. Group B streptococcal infection in older patients. Spectrum of disease and management strategies. *Drugs Aging* **6**:293–300, 1995.
4. Garcia-Lechuz JM, Bachiller P, Vasallo FJ, Munoz P, Padilla B, Bouza E. Group B streptococcal osteomyelitis in adults. *Medicine* (Baltimore) **78**:191–199, 1999.
5. Gardam MA, Low DE, Saginur R, Miller MA. Group B streptococcal necrotizing fasciitis and streptococcal toxic shock-like syndrome in adults. *Arch Intern Med* **158**:1704–1708, 1998.
6. Muñoz P, Llancaqueo A, Rodriguez-Créixems M, Peláez T, Martin L, Bouza E. Group B streptococcus bacteremia in nonpregnant adults. *Arch Intern Med* **157**:213–216, 1997.
7. Terry SM, Ryan PE Jr. Penetrating mitral valve annular abscess. *J Heart Valve Dis* **6**:621–624, 1997.
8. Trivalle C, Martin E, Martel P, Jacque B, Menard JF, Lemeland JF. Group B streptococcal bacteraemia in the elderly. *J Med Microbiol* **47**:649–652, 1998.
9. Colford JM Jr, Mohle-Boetani J, Vosti KL. Group B streptococcal bacteremia in adults. Five years' experience and a review of the literature. *Medicine* (Baltimore) **74**:176–190, 1995.
10. Jackson LA, Hilsdon R, Farley MM, Harrison LH, Reingold

AL, Plikaytis BD, Wenger JD, Schuchat A. Risk factors for group B streptococcal disease in adults. *Ann Intern Med* **123**:415–420, 1995.

11. Opal SM, Cross A, Palmer M, Almazan R. Group B streptococcal sepsis in adults and infants. Contrasts and comparisons. *Arch Intern Med* **148**:641–645, 1988.
12. Schwartz B, Schuchat A, Oxtoby MJ, Cochi SL, Hightower A, Broome CV. Invasive group B streptococcal disease in adults. A population-based study in metropolitan Atlanta. *J Am Med Assoc* **266**:1112–1114, 1991.
13. Casey JI, Maturlo S, Albin J, Edberg SC. Comparison of carriage rates of group B streptococcus in diabetic and nondiabetic persons. *Am J Epidemiol* **116**:704–708, 1982.
14. Piper JM, Georgiou S, Xenakis EM, Langer O. Group B streptococcus infection rate unchanged by gestational diabetes. *Obstet Gynecol* **93**:292–296, 1999.
15. Raimer K, O'Sullivan MJ. Influence of diabetes on group B *Streptococcus* colonization in the pregnant patient. *J Matern Fetal Med* **6**:120–123, 1997.
16. Tebbs SE, Gonzalez AM, Wilson RM. The role of aldose reductase in diabetic neutrophil phagocytosis and killing. *Clin Exp Immunol* **84**:482–487, 1991.
17. Boland OM, Blackwell CC, Clarke BF, Ewing DJ. Effects of ponalrestat, an aldose reductase inhibitor, on neutrophil killing of *Escherichia coli* and autonomic function in patients with diabetes mellitus. *Diabetes* **42**:336–340, 1993.
18. Lin X, Candlish JK, Thai AC. Superoxide production by neutrophils from diabetics and normal subjects in response to glucose and galactose. *Exp Mol Pathol* **58**:229–236, 1993.
19. Rajasoorya C, Wong SF, Chew LS. Diabetic ketoacidosis—A study of 33 episodes. *Singapore Med J* **34**:381–384, 1993.
20. Junger WG, Hoyt DB, Davis RE, Herndon-Remelius C, Namiki S, Junger H, Loomis W, Altman A. Hypertonicity regulates the function of human neutrophils by modulating chemoattractant receptor signaling and activating mitogen-activated protein kinase p38. *J Clin Invest* **101**:2768–2779, 1998.
21. Edwards MS, Baker CJ, Kasper DL. Opsonic specificity of human antibody to the type III polysaccharide of group B *Streptococcus*. *J Infect Dis* **140**:1004–1008, 1979.
22. Kasper DL, Paoletti LC, Wessels MR, Guttormsen H-K, Carey VJ, Jennings HJ, Baker CJ. Immune response to type III group B streptococcal polysaccharide-tetanus toxoid conjugate vaccine. *J Clin Invest* **98**:2308–2314, 1996.
23. Guttormsen H-K, Baker CJ, Edwards MS, Paoletti LC, Kasper DL. Quantitative determination of antibodies to type III group B streptococcal polysaccharide. *J Infect Dis* **173**: 142–150, 1996.
24. Anderson DC, Edwards MS, Baker CJ. Luminol-enhanced chemiluminescence for evaluation of type III group B streptococcal opsonins in human sera. *J Infect Dis* **141**:370–381, 1980.
25. Wilson RM, Tomlinson DR, Reeves WG. Neutrophil sorbitol production impairs oxidative killing in diabetes. *Diabetes Med* **4**:37–40, 1987.
26. Tebbs SE, Lumbwe CM, Tesfaye S, Gonzalez AM, Wilson RM. The influence of aldose reductase on the oxidative burst in diabetic neutrophils. *Diabetes Res Clin Pract* **15**:121–129, 1992.
27. MacRury SM, Gemmel CG, Paterson KR, MacCuish AC. Changes in phagocytic function with glycaemic control in diabetic patients. *J Clin Pathol* **42**:1143–1147, 1989.
28. Demerdash TM, Seyrek N, Smogorzewski M, Marcinkowski W, Nasser-Moadelli S, Massry SG. Pathways through which glucose induces a rise in $[Ca^{2+}]i$ of polymorphonuclear leukocytes of rats. *Kidney Int* **50**:2032–2040, 1996.
29. Repine JE, Clawson CC, Goetz FC. Bactericidal function of neutrophils from patients with acute bacterial infections and from diabetics. *J Infect Dis* **142**:869–875, 1980.
30. McMahon MM, Bistrian BR. Host defenses and susceptibility of infection in patients with diabetes mellitus. *Infect Dis Clin North Am* **9**:1–9, 1995.
31. Saeed FA, Castle GE. Neutrophil chemiluminescence during phagocytosis is inhibited by abnormally elevated levels of acetoacetate: Implications for diabetic susceptibility to infections. *Clin Diagn Lab Immunol* **5**:740–743, 1998.
32. Margni RA, Malan Borel I. Paradoxical behavior of asymmetric IgG antibodies. *Immunol Rev* **163**:77–87, 1998.
33. Alexiewicz JM, Kumar D, Smogorzewski M, Klin M, Massry SG. Polymorphonuclear leukocytes in non-insulin dependent diabetes mellitus: Abnormalities in metabolism and function. *Ann Intern Med* **123**:919–924, 1995.
34. Nielson CP, Hindson DA. Inhibition of polymorphonuclear leukocyte respiratory burst by elevated glucose concentrations *in vitro*. *Diabetes* **38**:1031–1035, 1989.
35. Ihm SH, Yoo HJ, Park SW, Park CJ. Effect of tolrestat, an aldose reductase inhibitor, on neutrophil respiratory burst activity in diabetic patients. *Metabolism* **46**:634–638, 1997.
36. Fóris G, Paragh G, Dezsõ B, Keresztes T, Balogh Z, Szabó J. Altered postreceptor signal transduction of formyl-Met-Leu-Phe receptors in polymorphonuclear leukocytes of patients with non-insulin-dependent diabetes mellitus. *Clin Immunol Immunopathol* **86**:95–101, 1998.
37. Kiersztejn M, Chervu I, Smogorzewski M, Fadda GZ, Alexiewicz JM, Massry SG. On the mechanism of impaired phagocytosis in phosphate depletion. *J Am Soc Nephrol* **2**:1484–1489, 1992.
38. Seyrek N, Marcinkowski W, Smogorzewski M, Demerdash TM, Massry SG. Amlodipine prevents and reverses the elevation in $[Ca^{2+}]i$ and the impaired phagocytosis of PMNL of diabetic rats. *Nephrol Dial Transplant* **12**:265–272, 1997.
39. Edwards MS, Fuselier PA. Enhanced susceptibility of mice with streptozotocin-induced diabetes to type II group B streptococcal infection. *Infect Immun* **39**:580–585, 1983.